

Inhibitors of Src kinase for use in Alzheimer's disease

The present invention relates to the identification of inhibitors of Src kinase, assays and methods useful for such identification and the use of Src inhibitors for the preparation of pharmaceuticals for the treatment of Alzheimer's disease.

Alzheimer's disease is a neurodegenerative disorder of the brain, which is accompanied at the cellular level by a massive loss of neurons in the limbic system and in the cerebral cortex. In the brain areas affected, protein deposits, so-called plaques, can be detected at the molecular level, which are an essential characteristic of Alzheimer's disease. The protein occurring most frequently in these plaques is a peptide of 40 to 42 amino acids in size, which is designated as A β -peptide. This peptide is a cleavage product of a larger protein of 695 to 770 amino acids, the so-called Amyloid Precursor Protein (APP).

APP is an integral transmembrane protein, which firstly traverses the lipid bilayer. By far the largest part of the protein is extracellular, while the shorter C-terminal domain is directed into the cytosol (Figure 1). APP is the substrate of three different proteases: α -secretase, β -secretase and γ -secretase. Within APP, about two thirds of the A β -peptide originates from the extracellular domain and about one third from the transmembrane domain.

Beside the membrane-linked APP, a secreted form of APP can be detected which consists of the large ectodomain of APP and is designated as APP_{sec} ("secreted APP"). APP_{sec} is formed from APP by proteolytic cleavage, which is effected by the α -secretase (Figure 2). This proteolytic cleavage takes place within the amino acid sequence of the A β -peptide (after amino acid residue 16 of the A β -peptide). Proteolysis of APP by the α -secretase thus excludes the formation of the A β -peptide.

The A β -peptide can thus only be formed from APP in an alternative processing route. It is postulated that two proteases are involved in this processing, one protease, which is designated as β -secretase, cleaving at the
 5 N-terminus of the A β -peptide in the APP and the second protease, which is designated as γ -secretase, releasing the C-terminus of the A β -peptide (Kang, J. et al., (1987) Nature, 325, 733 (Figure 2).

There are many indications that the A β -peptide is a crucial factor in the
 10 occurrence of Alzheimer's disease. Inter alia, neurotoxicity of A β -fibrils in cell culture is postulated (Yankner, B.A. et al., (1990) Proc Natl Acad Sci USA, 87, 9020). In patients with Down's syndrome, in which APP occurs in an additional copy, the neuropathology characteristic of Alzheimer's disease also occurs even at an age of 30 years. Here, it is assumed that the
 15 overexpression of APP follows an increased conversion into the A β -peptide (Rumble, B. et al., (1989), N. Engl. J. Med., 320, 1446).

Furthermore the strongest indication of the central role of the A β -peptide in Alzheimer's disease are the familial forms of the disease. Here, mutations are
 20 found in the APP gene around the area of the secretase cleavage sites or in two further AD-associated genes (presenilins), which in cell culture lead to a significant increase in A β production (Scheuner, D. et al., (1996), Nature Medicine, 2, 864).

25 There are a number of indications that APP is first cleaved by the β -secretase, to serve thereafter as a substrate for γ -secretase (Maruyama, K. Y. et al., (1994) Biochem. Biophys Res Commun, 202, 1517; Estus, S. et al., (1992), Science, 255, 726). The γ -secretase therefore has a crucial role in the formation of the A β -peptide. A demonstration of the activity of the γ -
 30 secretase which is customarily used is the detection of the A β -peptide, which, however, frequently turns out to be difficult.

An important reason for this is that only a small part of APP is converted into the A β -peptide (Simons M, et al., *Neurosci* (1996) 1;16(3):899-908). Moreover, the A β -peptide is a small breakage fragment of about 4 kDa and, on account of its hydrophobic character, has a great tendency to self-aggregation so that it easily precipitates under physiological conditions (Hilbich, C. et al., (1991) *J. Mol. Biol.*, 218, 149).

The short, 47 amino acid-long C-terminal tail of APP is exposed to the cytosol and is the site of interaction for molecular adaptors containing PTB domains, named Fe65, X11, m-dab and Jip1. Since the binding of these proteins is dependent on the YENPTY sequence on APP, it is expected that their interactions are not simultaneous (T. Russo et al. (1998) *FEBS Lett.* 434, 1-7; N. Zambrano et al. (1997) *J. Biol. Chem.* 272, 6399-6405). Indeed, it has been shown, in cultured cells, that Fe65 and X11 proteins have opposite effects on the proteolytic processing of APP (Sabo S. et al. (1999) *J. Biol. Chem.* 274, 7952-7957; Borg J.P. et al. (1998) *J. Biol. Chem.* 273, 14761-14766). A possible explanation for these findings may reside in the recruitment, by either Fe65 or X11, of APP in different macromolecular complexes, depending on the interaction of different sets of proteins with the other protein-protein interaction domains of the two adaptors.

The APP cytodomain contains various serine and threonine residues, which are phosphorylated *in vitro* by different kinase activities. Thr668 is a main site of phosphorylation *in vivo*, being phosphorylated by neuronal cyclin-dependent kinases, Cdk5 in neurons (Iijima, K. et al. (2000) *J. Neurochem.* 75, 1085-1091), cdc2 kinase in dividing cells (Suzuki, T. et al. (1994) *EMBO J.* 13, 1114-1122 ; Oishi, M. et al. (1997) *Mol. Med.* 3, 111-123), and glycogen synthase kinase 3b (GSK3b) and stress-activated protein kinase 1b (SAP kinase1b) *in vitro* (Aplin, E. E. et al. (1996) *J. Neurochem.* 67, 699-707 ; Standen, C. L. et al. (2001) *J. Neurochem.* 76, 316-320). It has been shown

that phosphorylation of APP regulates neurite extension in differentiating PC12 cells (Ando, K. et al. (1999) J. Neurosci. 19, 4421-4427), while, at the molecular level, APP phosphorylation may regulate the binding to the PTB domain-containing adaptors and its processing (Ando K. et al. (2001) J Biol. Chem., 276, 40353-40361).

Previously it was demonstrated that APP is phosphorylated at tyrosine 682 in cells expressing an active form of the Abl non receptor tyrosine kinase (Zambrano N. et al. (2001) J Biol. Chem. 276, 19787-92); active Abl is recruited in proximity to APP by Fe65, which may bind simultaneously these two proteins through its WW domain (Abl) and PTB2 domain (APP). Phosphorylation of Tyr682 of APP generates a docking site for the SH2 domain of Abl and, in fact stable complexes between APP and Abl are formed.

In order to test the hypothesis that tyrosine phosphorylation regulates pp60c-src biological activity, Kmiecik and Shalloway (1987) Cell 49, 65-73 described the construction and study of pp60c-src mutants in which Tyr 527 and Tyr 416 were separately or coordinately altered to phenylalanine. Tyr----Phe 527 mutation strongly activated pp60c-src transforming and kinase activities, whereas the additional introduction of a Tyr----Phe 416 mutation suppressed these activities. Tyr----Phe 416 mutation of normal pp60c-src eliminated its partial transforming activity, which suggests that transient or otherwise restricted phosphorylation of Tyr 416 is important for pp60c-src function even though stable phosphorylation is not observed in vivo. Normally, pp60c-src is phosphorylated in vivo at tyrosine 527, a residue not present in pp60v-src (its transforming homolog), and not at tyrosine 416, its site of in vitro autophosphorylation.

Williamson et al. (2002) J. Neurochem. 22, 10-20) described the rapid phosphorylation of neuronal proteins including Tau and Focal adhesion

kinase in response to amyloid- β peptide exposure and an involvement of Src family protein kinases.

Slack, B. E. and Berse; B. (1998) described in Society for Neuroscience Abstracts, Vol. 24, No. 1-2, pp. 208 (Conference/Meeting Information: 28th Annual Meeting of the Society for Neuroscience, Part 1, Los Angeles, California, USA, Nov. 7-12, 1998 Society for Neuroscience, ISSN: 0190-5295) a role for tyrosine kinases in the stimulation of APP release by action of muscarinic m3 acetylcholine receptors. They described a role for Src tyrosine kinase in the regulation of APP_{sec} release by muscarinic receptors. They demonstrated that an increase in the active form of Src leads to a decrease in secAPP.

The present invention provides methods of identifying therapeutic agents for the treatment of Alzheimer's disease.

One embodiment of the invention provides methods for identifying inhibitors of Src activity, whereby the methods comprise the steps

- a) providing a Src protein (i.e. a Src encoding DNA under control of an expression element) and
- b) determining if a compound inhibits the activity of Src.

The Src protein could be any mammalian Src protein, preferably human Src (SEQ ID NO. 1 (isoform 1) and SEQ ID NO. 2 (isoform 2)) or rodent Src (SEQ ID NO. 3). The protein could be obtained by expressing human Src or rodent Src cDNA, by using e. g. sequences SEQ ID NO. 4 or SEQ ID NO. 5. In addition, one of the sequences deposited under Genbank Accession No. M17031 or BC011566 can be used.

For the method preferably a mammalian cell or cell line is used in which Src is expressed, either naturally or because the cell / cell line is genetically engineered. In a particular embodiment primary cultures of neurones are used.

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Another embodiment of the invention relates to a method of identifying compounds, which inhibit Src expression. The method comprises the steps

- 10 a) providing a sequence which regulates Src expression (i.e. a Src promotor sequence) and
- b) determining if a compound inhibits the expression of Src protein.

The sequence which regulates Src expression is preferably linked to a reporter gene or a reporter gene construct. Such reporter gene can easily be
15 used to determine, if a compound inhibits the expression of Src. Another possibility is the use of the Src gene. The region of chromosome 20 including the Src gene is deposited under Genbank Accession no AL133293 .

An house-keeping promoter, which can be used as a sequence which
20 regulates Src expression, has been described, as well as an alternative promoter regulated by the Hepatic Nuclear Factor-1a (Bonham et al. (2000) J. Biol. Chem. 275, 37604) (Genbank accession number AF272982).

Active compounds, this means compounds, which inhibit Src expression or
25 which inhibit Src protein activity, can be used as pharmaceuticals. Such compounds can be used for the preparation of a pharmaceutical for the treatment or prevention of Alzheimer's disease.

Another embodiment of the invention relates to the use of compounds identified by one of the forgoing methods for preparing a pharmaceutical for
30 the treatment of Alzheimer's disease.

The invention further relates to a method of preparing a pharmaceutical for treatment of Alzheimer's disease comprising the steps

- 5 a) identification of a therapeutic compound by the use of one of the methods described above;
- b) optimisation of the identified compound; and
- c) formulation of the optimised compound.

10 Another embodiment of the invention relates to the use of Src inhibitors known in the art for the preparation of a pharmaceutical for the treatment or the prevention of Alzheimer's disease. The invention relates to the use of PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine) as pharmaceutical. The invention further relates to the use of PP2 for the preparation of a pharmaceutical for the treatment of Alzheimer's disease.

15 Another embodiment of the invention refers to the use of PP2 for the preparation of a pharmaceutical for the treatment of Alzheimer's disease.

20 The new results demonstrate, that APP is phosphorylated not only by Abl. In fact Src kinase is also responsible for APP tyrosine phosphorylation. It is expected that tyrosine phosphorylation of APP alters the binding properties of its cytodomain, and then the processing of APP could be consequently modified. If so, this will prove useful the use of currently available tyrosine kinase inhibitors, and to design new ones for pharmacological modulation of the release of APP processing products in cellular and animal models of Alzheimer's disease.

25 Taken together, the results from the following examples suggest that exogenous Src activity is responsible for activation of a pathway resulting in increased A β secretion. This also suggests that PP2 could be used as a pharmacological agent able to reduce A β secretion in cellular and animal systems resembling AD status.

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Therefore, a line of CHO cells stably overexpressing APP751 form was used, which secretes discrete levels of A β . If secretion involves endogenous Src activity, PP2 should be able to inhibit production of A β also in this cellular system. This is indeed the case since, as shown in Figure 6, PP2 reduces in a dose-dependent manner the amount of A β at the three time points tested (1, 3 and 12 hours), while PP3, used at the highest concentration, has no effect.

These experiments clearly outline the relevance of Src activation in A β secretion and constitute the basis to try pharmacological applications for tyrosine kinase inhibitors in the treatment of AD. Screening of such inhibitors and their optimization will lead to the development of potential, innovative therapeutic agents for treatment of Alzheimer's disease.

15 Examples

Example 1

Cell culture, transfections and treatments

Human embryonic kidney cells (HEK293) were cultured in Dulbecco's modified minimal medium supplemented with 10% fetal calf serum at 37 °C in a 5% CO₂ atmosphere. For transfection, cells were grown for 16 hours in antibiotic-free medium, and transfected with Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. The total amount of DNA was maintained constant by addition of empty vector DNA. 48 hours after transfection, culture medium were recovered, while cells were harvested in ice-cold phosphate buffer saline (PBS) and gently lysed in lysis buffer (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 10% glycerol, 50 mM NaF, 1mM Na vanadate), in the presence of a protease inhibitor cocktail (Complete, EDTA-free, Roche). The extracts were clarified by centrifugation at 16,000 x g at 4 °C, and the protein concentration determined by the Bio-

Rad protein assay according to manufacturer's instructions. For metabolic labelling, 36 hours after transfection, cells were incubated for 30 minutes in medium without methionine and cysteine, then the medium was replaced with fresh medium containing 80 $\mu\text{Ci/ml}$ of ^{35}S -methionine/cysteine mixture (Promix, 1000 Ci/mmol, Amersham Pharmacia Biotech). Pulse was for 30 minutes, then the radioactive medium was replaced with complete medium for 90 minutes. CHO cells expressing wild-type APP751 have been described (N. Zambrano et al. (1997) J. Biol. Chem. 272, 6399-6405).

Immuno-precipitations were performed either on culture medium, or on cellular lysates with 1 $\mu\text{g/sample}$ of the 6E10 monoclonal antibody (Signet). Radioactive samples were resolved on 10% SDS-PAGE and quantitated with a Storm 840 phosphorimager (Amersham Pharmacia Biotech).

PP2 and PP3 (Hanke, J.H. et al. (1996) J. Biol. Chem. 271, 695–701) were purchased from Calbiochem and dissolved in DMSO; treatments were performed on transfected cells for 48 hours with vehicle alone, or with 1, 5 and 20 μM PP2 or PP3. Concentration of DMSO was the same in all samples. Treatments on CHO/APP751 cells was made with DMSO, or PP2 (5 and 20 μM), or PP3 (20 μM).

Example 2

HEK293 cells were transfected with APP695 expression vectors. This cellular system was used because these cells are transfected to high efficiency, and because they express the whole set of processing activities required for APP maturation. In fact, upon transfection of human APP695 expression vectors, both α -secretase product (APP_{sec}) and β - γ -secretase products ($\text{A}\beta$) accumulate in the culture medium. HEK293 cells were co-transfected with APP and either empty vector, or active Abl (Abl-PP), or active Src (SrcYF) kinases, and the secretion of APP_{sec} and $\text{A}\beta$ were evaluated. Transfected cells have been pulse-labelled with ^{35}S -Met/Cys mix for 30', and chased with cold amino acids for 90'. Both medium and protein extracts have been used

in immunoprecipitation experiments for quantitative dosage of radiolabeled APP_{sec} and holo-APP, respectively. While the stability of holo-APP was not affected by co-transfection with either Abl-PP or SrcYF active kinases (Figure 3A), a reduction in the relative amounts of APP_{sec}, normalised to the
 5 corresponding holo-APP synthesised during the pulse period was observed in both cases (Figure 3B). This can be interpreted as a decrease of the activity of the α -secretase pathway upon co-transfection of APP with the active kinases.

10 Example 3

Next, the accumulation of A β from cells transfected as above was investigated. The dosage was performed by sandwich ELISA assays on culture medium at 48 hours after transfection (Figure 4). The assay clearly
 15 shows that in Src-expressing cells the amount of A β secreted is more elevated than in the presence of the control vector, or Abl-PP.

The mechanism by which active Src elicits such a dramatic increase in A β secretion is not clear. However, it might depend on phosphorylation of some other proteins, different from APP, since tyrosine phosphorylation of APP by
 20 Abl-PP does not result in increased A β levels. Accordingly, a similar behaviour is obtained with various APP mutants bearing tyrosine substitutions to either glycine or phenylalanine (data not shown).

Anyway, the rise in A β levels does depend on the tyrosine kinase activity of Src, since the accumulation of A β is sensitive to treatment by the Src-family
 25 specific inhibitor PP2. In fact, as shown in Figure 5, the exposure of transfected cells for 48 hours to increasing concentrations of PP2 results in dose-dependent decrease of secreted A β . Either PP3, an inactive analog of PP2, or vehicle alone, do not affect A β rise by SrcYF transfection.

Taken together, these observations suggest that exogenous Src activity is responsible for activation of a so far unidentified pathway resulting in increased A β secretion.

5 REFERENCES

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- 2) N. Zambrano et al. (1997) J. Biol. Chem. 272, 6399-6405.
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- 12) Zambrano N. et al. (2001) J Biol Chem 276, 19787-19792.
- 13) Hanke, J.H. et al. (1996) J. Biol. Chem. 271, 695-701.

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Table 1:

Protein sequence of isoform 1 of human Src protein (SEQ ID NO. 1)

MGSNKS PKDASQRRRSLEPAENVHGAGGGAFFASQTPSKPASADGHRGPSAAFAPAAA
 25 EPKLFGGFNSSDTVTSPQRAGPLAGGVTTFFVALYDYESRTETDLSFKKGERLQIVNNTRKVD
 VREGDWWLAHSLSTGQTGYIPSNYVAPSDSIQAEWYFGKITRRESERLLLNAENPRGTFL
 VRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDGGFYITSRTQFNSLQQLVAYYSKHAD
 GLCHRLTTVCPTSKPQTQGLAKDAWEIPRESLRLEVKLGGCFGEVWMGTWNGTTRVAIK
 TLKPGTMSPEAFLQEAQVMKKLRHEKLVQLYAVVSEPIYIVTEYMSKGSLLDFLKGETGKYL

RLPQLVDMAAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNEYTARQ
 GAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGMVNREVLDQVERGYRMP
 CPPECPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEPQYQPGENL

5 Table 2:

Protein sequence of isoform 2 of human Src (SEQ ID NO. 2)

MGSNKS PKDASQRRRSLEPAENVHGAGGGAFFASQTPSKPASADGHRGPSAAFAPAAA
 EPKLFGGFNSSDTVTSPQRAGPLAGGVTTFVALYDYESRTETDLSFKKGERLQIVNNTGEGD
 10 WWLAHSLSTGQTGYIPSNYVAPSDSIQAEWYFGKITRRESERLLLNAENPRGTFLVRESET
 TKGAYCLSVSDFDNAKGLNVKHYKIRKLDSSGGFYITSRTQFNSLQQLVAYYSKHADGLCHRL
 TTVCP TSKPQTQGLAKDAWEIPRESLRLEV KLGQCGFGEVWMGTWNGTTRVAIKTLKPGT
 MSPEAFLQEAQVMKKLRHEKLVQLYAVVSEEPYIVTEYMSKGSLLDFLKGETGKYLRPLQL
 VDMAAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNEYTARQGAKF
 15 PIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGMVNREVLDQVERGYRMPCPPE
 CPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEPQYQPGENL

Table 3:

Sequence of mouse Src protein (SEQ ID NO. 3)

20

MGSNKS PKDASQRRRSLEPSENVHGAGGGAFFASQTPSKPASADGHRGPSAAFVPPAAEP
 KLFGGFNSSDTVTSPQRAGALAGGVTTFVALYDYESRTETDLSFKKGERLQIVNNTRKVDVR
 EGDWWLAHSLSTGQTGYIPSNYVAPSDSIQAEWYFGKITRRESERLLLNAENPRGTFLVR
 ESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSSGGFYITSRTQFNSLQQLVAYYSKHADGL
 25 CHRLTTVCPTSKPQTQGLAKDAWEIPRESLRLEV KLGQCGFGEVWMGTWNGTTRVAIKTLK
 PGTMSPEAFLQEAQVMKKLRHEKLVQLYAVVSEEPYIVTEYMNKGSLLDFLKGETGKYLRRL

PQLVDMSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGGLARLIEDNEYTARQG
 AKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTKGRVPYPGMVNREVLQDQVERGYRMPC
 PPECPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEPQYQPGENL

5 Table 4:

cDNA sequence encoding human Scr protein (SEQ ID NO. 4)

1 catcgagggtt ttgagaggct aactctccca aaaaggacca tgggtagcaa
 10 caagagcaag
 61 cccaaggatg ccagccagcg gcgccgcagc ctggagcccg ccgagaacgt
 gcacggcgct
 121 ggcgggggcg ctttccccgc ctgcagacc ccagcaagc cagcctcggc
 cgacggccac
 15 181 cgcgggccca gcgcggcctt cgccccgcg gccgcccagc ccaagctggt
 cggaggcttc
 241 aactcctcgg acaccgtcac ctccccgcag agggcgggcc cgctggccgg
 tggagtgacc
 301 acctttgtgg ccctctatga ctatgagtct aggacggaga cagacctgtc
 20 cttcaagaaa
 361 ggcgagcggc tccagattgt caacaacaca gaggagact ggtggctggc
 ccactcgctc
 421 agcacaggac agacaggcta catccccagc aactacgtgg cgccctccga
 ctccatccag
 25 481 gctgaggagt ggtattttgg caagatcacc agacgggagt cagagcggtt
 actgctcaat
 541 gcagagaacc cgagaggac cttcctcgtg cgagaaagtg agaccacgaa
 aggtgcctac
 601 tgctctcag tgtctgactt cgacaacgcc aaggcctca acgtgaagca
 30 ctacaagatc
 661 cgcaagctgg acagcggcgg cttctacatc acctcccgca ccagttcaa
 cagcctgcag
 721 cagctggtgg cctactactc caaacacgcc gatggcctgt gccaccgct
 caccaccgtg
 35 781 tgccccacgt ccaagccgca gactcagggc ctggccaagg atgcctggga
 gatccctcgg

841 gagtcgctgc ggctggaggt caagctgggc cagggtgct ttggcgaggt
 gtggatggg
 901 acctggaacg gtaccaccag ggtggccatc aaaaccctga agcctggcac
 gatgtctcca
 5 961 gaggccttcc tgcaggaggc ccaggatcag aagaagctga ggcatgagaa
 gctggtgcag
 1021 ttgtatgctg tggtttcaga ggagccatt tacatcgtca cggagtacat
 gagcaagggg
 1081 agtttgctgg actttctcaa gggggagaca ggcaagtacc tgcggctgcc
 10 tcagctgggtg
 1141 gacatggctg ctcagatcgc ctcaggcatg gcgtacgtgg agcggatgaa
 ctacgtccac
 1201 cgggaccttc gtgcagccaa catcctggtg ggagagaacc tgggtgtgcaa
 agtggccgac
 15 1261 tttgggctgg ctcggctcat tgaagacaat gagtacacgg cgcggaagg
 tgccaaattc
 1321 cccatcaagt ggacggctcc agaagctgcc ctctatggcc gcttcacat
 caagtcggac
 1381 gtgtggctct tcgggacct gctgactgag ctcaccacaa agggacgggt
 20 gccctaccct
 1441 gggatggtga accgcgaggt gctggaccag gtggagcggg gctaccggat
 gccctgccc
 1501 ccggagtgtc ccgagtcct gcacgacctc atgtgccagt gctggcggaa
 ggagcctgag
 25 1561 gagcggccca ccttcgagta cctgcaggcc ttcttgagg actacttca
 gtccaccgag
 1621 cccagtagc agcccgggga gaacctctag gcacaggcgg gccagaccg
 gcttctcggc
 1681 ttggatcctg ggctgggtgg ccctgtctc ggggcttgcc ccactctgcc
 30 tgctgctgt
 1741 tggtcctctc tctgtggggc tgaattgcca ggggcgaggc ccttctctt
 tgggtggcatg
 1801 gaaggggctt ctggacctag ggtggcctga gagggcgggt ggtatgcgag
 accagcacgg
 35 1861 tgactctgtc cagctcccgc tgtggccgca cgctctccc tgactcct
 cctggagctc
 1921 tgtgggtctc tggaagagga accaggagaa gggctggggc cggggctgag
 ggtgccctt

1981 tccagcctca gcctactccg ctactgaac tccttcccca cttctgtgce
 acccccgggc
 2041 tatgtcgaga gctggccaaa gagcctttcc aaagaggagc gatgggccc
 tggccccgc
 5 2101 tgctgccac cctgcccctt gccatccatt ctggaaacac ctgtaggcag
 aggctgccga
 2161 gacagaccct ctgccgtgc ttccaggctg ggcagcaca ggccttgct
 ggcctgatga
 2221 tgggtgggtgg gtgggatgag taccctcca aacctgccc tccttagacc
 10 tgagggaccc
 2281 ttcgagatca tcttctctt gccccattt caccatggg gagacagt
 agagcgggga
 2341 tgtgacatgc ccaaggccac ggagcagttc agagtggagg cgggcttga
 acccggtgct
 15 2401 ccctctgtca tcctcaggaa ccaacaattc gtcggaggca tcatggaaag
 actgggacag
 2461 cccaggaaac aaggggtctg aggatgcatt cgagatggca gattccact
 gccgtgccc
 2521 gctcagccca gctgttggga acagcatgga ggcagatgtg gggctgagct
 20 ggggaatcag
 2581 ggtaaaaggc gcaggtgtgg agagagaggc ttcaatcggc ttgtgggtga
 tgtttgacct
 2641 tcagagccag ccggtatga aaggagcga gccctcggc tctggaggca
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 2821 ggagggaaaa gattgcctaa gcgggggtga aagaggacgt gttaccact
 30 gccatgcacc
 2881 aggactggct gtgtaacctt ggggtggccc tgctgtctct ctgggctgca
 gactctgcc
 2941 cacatgtggc catggcctct gcaactgctc agctctggc caggccctgt
 ggcaggacac
 35 3001 acatggtgag cctagccctg ggacatcagg agactgggct ctggctctgt
 tcggcctttg
 3061 ggtgtgtggt ggattctccc tgggcctcag tgtgcccac tgtaaaggg
 cagctgacag

3121 tttgtggcat cttgccaagg gtccctgtgt gtgtgtatgt gtgtgcatgt
 gtgcgtgtct
 3181 ccatgtgcgt ccatatttaa catgtaaaaa tgtccccccc gtcctgcccc
 ccaaacatgt
 5 3241 tgtacatttc accatggccc cctcatcata gcaataacat tcccactgcc
 aggggttctt
 3301 gagccagcca ggccctgcca gtggggaagg aggccaagca gtgcctgcct
 atgaaatttc
 3361 aacttttctt ttcatacgtc tttattaccc aagtcctctc cctccattc
 10 cagtcaaate
 3421 tgggctcact caccacagcg agctctcaaa tccctctcca actgcctaag
 gccctttgtg
 3481 taagggtgtt taatactgtc cttttttttt ttttaacagt gttttgtaga
 tttcagatga
 15 3541 ctatgcagag gcctggggga cccctggctc tgggccgggc ctggggctcc
 gaaattccaa
 3601 ggcccagact tgcggggggg ggggggggtat ccagaattgg ttgtaaatac
 tttgcatatt
 3661 gtctgattaa acacaaacag acctcagaaa aaaaaaaaaa aaaaaaaaaa a
 20

Table 5:

25 Mouse Src cDNA sequence (SEQ ID NO. 5)

1 atgggcagca acaagagcaa gcccaaggac gccagccagc ggcgccgcag
 cctggagccc
 61 tcggaaaacg tgcacggggc agggggcgcc ttcccggcct cacagacacc
 30 gagcaagccc
 121 gcctccgccc acggccaccg cgggccagc gccgccttcg tgccgcccgc
 ggccgagccc
 181 aagctcttcg gaggttcaa ctctcggac accgtcacct ccccgagag
 ggcgggcgct
 35 241 ctggcaggtg gggtgaccac ctttgtggcc ctctatgact atgagtcag
 gacagagact
 301 gacctgtcct tcaagaaagg ggagcggctg cagattgtca ataacacgag
 gaaggtggat

361 gtcagagagg gagactggtg gctggcacac tcgctgagca cgggacagac
 cggttacatc
 421 cccagcaact atgtggcgcc ctccgactcc atccaggctg aggagtggta
 ctttggcaag
 5 481 atcactagac gggaatcaga gcggtgctg ctcaacgccg agaaccgag
 agggaccttc
 541 ctcgtagagg agagtgagac cacaaaaggt gcctactgcc tctctgtatc
 cgacttcgac
 601 aatgccaagg gcctaaatgt gaaacactac aagatccgca agctggacag
 10 cggcggtttc
 661 tacatcacct cccgcacca gttcaacagc ctgcagcagc tcgtggctta
 ctactccaaa
 721 catgctgatg gcctgtgtca ccgctcact accgtatgtc ccacatccaa
 gcctcagacc
 15 781 cagggattgg ccaaggatgc gtgggagatc ccccgaggat ccctgcggct
 ggaggtcaag
 841 ctgggccagg gttgcttcgg agaggtgtgg atggggacct ggaacggcac
 cagaggggtt
 901 gccatcaaaa ctctgaagcc aggcaccatg tccccagagg ccttctgtga
 20 ggaggcccaa
 961 gtcataaga aactgaggca cgagaaactg gtgcagctgt atgctgtggt
 gtcggaagaa
 1021 cccatttaca ttgtgacaga gtacatgaac aaggggagtc tgctggactt
 tctcaagggg
 25 1081 gaaacgggca aatatttgcg gctaccccag ctggtggaca tgtctgtctc
 gatcgcttca
 1141 ggcattggct atgtggagcg gatgaactat gtgcaccggg accttcgagc
 cgccaatatc
 1201 ctatgtaggg agaacctggt gtgcaaagtg gccgactttg gggtggcccc
 30 gctcatagaa
 1261 gacaacgaat acacagcccc gcaagggtgcc aaattcccca tcaagtggac
 cgcccctgaa
 1321 gctgctctgt acggcagggt caccatcaag tcggatgtgt ggtcctttgg
 gattctgctg
 35 1381 accgagctca ccactaaggg aagagtgcc tatcctggga tggatgaaccg
 tgaggttctg
 1441 gaccaggtgg agcggggcta ccggatgcct tgtcccccg agtgccccga
 gtccttgcac

1501 gaccttatgt gccagtgctg gcggaaggag cccgaggagc ggcccacctt
cgagtacctg

1561 caggccttcc tggaagacta ctttacgtcc actgagccac agtaccagcc
cgggggagaac

5

1621 ctatag